

Analysis and Experiment of Centrifugal Force for Microfluidic ELISA CD Platform

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Abstract— This paper presents an analysis and experiment of centrifugal force for microfluidic Enzyme-Linked Immunosorbent Assay (ELISA) on a compact-disc (CD) platform. The ELISA CD was designed based on the centrifugal force as a driving force while capillary force acts as preventing barrier. The CD composed of 2 layers: the substrate layer is made of PMMA and the top layer is laminated with an Adhesive Sealing Films (ASF) or thermal seal. The PMMA substrate was fabricated by CNC micromachining. The ELISA CD consists of 7 reservoirs: waste, detection, serum, conjugate, washing solution, substrate and stopping solution. In the experiment, the reservoirs were filled with colour liquid and the fluid flow behaviour of the liquid in the CD was studied and monitored by a customized CD spin stand system equipped with visualization system. The experimental test results show an average of 9% error when compared with the theoretical calculation of burst frequency for all the reservoirs.

Keywords— Microfluidics, centrifugal force, Lab on CD, microsystem, ELISA, Burst frequency

I. INTRODUCTION

Anderson was the first person who developed the centrifugal fluidic system in 1969 and in many years this centrifugal microfluidics has been studied by many researchers[1-5] Centrifugal microfluidic system which is also known as Lab-on-a-compact disc (CD), has been commercialized for clinical

biochemistry for blood diagnostics and rapid immunoassays by companies Abaxis and Gyros, respectively [1]. Although components of microfluidic system: micropump, microvalve, microchannel, siphon, mixer, chamber and metering part are still under individual studies, in more advanced level, the integrated microfluidic system in different application is the topic of investigation for improving conventional bulky systems with low efficiency. Amongst all the most popular propulsion mechanisms (such as pneumatic, hydraulic, electrokinetic, thermal, acoustic, electrohydrodynamic, magnetic, chemical and electrochemical pump), centrifugal pumping benefits from being simple and inexpensive. Besides, centrifuge system is not dependent on pH, sample electrical, magnetic and ionic properties and fluid contact. Centrifugal microfluidic platform has been extensively studied in biology and chemistry because of versatility in manipulation of fluid, compatibility with wide range of sample, simple centrifugal pumping drive, and low fabrication expenses [2].

To control the liquid flow, microvalve plays the main role in the system. The accuracy of the result for the microfluidic system depends on it strongly. The valves are divided into two categories: active and passive valves. Passive valves, such as capillary valves, do not require any moving parts or external stimuli since their operation is dependent on the exploitation of generated surface tensions in the micro-domain. On the other hand, active valves (such as thermally actuated gels,

magnetically actuated ferrofluid, pneumatically controlled membranes, controlled surface wetting or electrokinetic valves) require external operation to actuate a physical gating mechanism [3]. To date, centrifugal microfluidic platforms largely use capillary valves to control the liquid flow within microchannels and at outlets of the CD reservoirs, since they are easy to implement in terms of cost and fabrication. Parallel testing system is required to conform the centrifugal microfluidic is operating as desired design for specific clinical diagnostic and pathogenic agent detection application. In addition lab on CD technique provides the ability to multiplex experiments, faster analysis times, fully automated processes, less reagent consumption, lower material cost, in comparison to conventional system [4]. The geometrical limitation of the microfluidic CD is the main disadvantage of the centrifugal system. However, this limitation is overcome by the discovery of a pneumatic pumping technique to pump fluids back from the rim (edge or CD) to the center of rotation (COR) [1]. Additionally, conventional computer CD ROM drive can be modified to be as the microfluidic CD reader with doing non-fundamental changes [5].

At present, the centrifugal microfluidic disc offers itself to be a promising solution for an integrated device that can: mix, dose (measure) and switch, decant, dilute, calibrate and separate liquids. Generally, the CD application in biology and microbiology is related mostly to Enzyme-Linked Immunosorbent Assay (ELISA) [6] and Nucleic acid analysis through Polymerase Chain Reaction (PCR) [7] and DNA hybridization [8,9]. Microfluidic CD system for ELISA mixes blood or plasma as sample, substrates, conjugates, washing buffers, primary antibodies, blocking proteins and antigen solution to receive final detectable signal for distinction between positive or negative testing sample. These materials are dictated to the system from biological and microbiological conventional assays. For PCR, mixing of the sample with the PCR reagent is followed by thermocycling to high temperature (95 °C) process and DNA amplification proves existence or inexistence of disease factor within the sample [10]. In previous studies, direct hemoglobin measurement and single step hematocrit determination are surveyed and fabricated on the microfluidic CDs [11,12]. In the other project, cultivation system of microorganism (*Caenorhabditis elegans*) has been studied in off-earth condition likes hyper- and hypo gravity and intense UV and gamma radiation. The feeding and waste removal process is the most critical issue and success of project gave opportunity for potential application of its achievement in investigation about the living creatures' behavior in the space mission and other planets [13]. The microfluidic CD not only can be widely used in biological application, but also it is a platform to monitor and survey about metallic pollution in environmental sample [14,15,16]. Monitoring allergic response of a patient to the cause of allergy is also made viable by the microfluidic CD [17]. MicroElectroMechanical System (MEMS) and biological-MEMS (BIOMEMS) parts and systems are fabricated in numerous different ways such as bulk micromachining with wet or dry etching, wafer bonding, deep reactive ion etching

(DRIE)[18]. The most favorable materials for microfabrication are polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), PolyCarbon (PC) and Cyclo-Olefin-Copolymer (COC). Polyurethane can be used for rapid prototyping and replication with master casting [19]. Hydrophobicity, the most important property related to the material, is dominant factor in the CD design. The PDMS and PC use mostly in CD fabrication are hydrophobic, so in cases that hydrophilic surface is required hydrophilic coating on hydrophobic surface and chemical or physical surface treatments (e.g. plasma treatment) can generate favorable surfaces.

Two critical issues in developing of microfluidic CD-based devices are microfabrication method and proper arrangement of the microfluidic components design for an application to take the best result [1]. This paper describes the fabrication method, analysis and experiment of a new design of microfluidic system for ELISA CD-based application.

II. CONCEPT OF ENTRIFUGAL MICROFLUIDICS

All aspects of fluid behavior is studied in fluid mechanics that energy, momentum and continuity equations govern whole system state. Laminar or turbulent, viscose or inviscid, compressible or incompressible, transient or fully developed flows are different condition of a real fluidic system. Simplifying assumptions are usually considered to cancel negligible terms in the equation of state. It is rational to interpret the centrifugal microfluidic system by incompressible Navier-Stokes equation [20].

$$\rho \left[\frac{du}{dt} + (u \cdot \nabla)u \right] = F - \nabla p + \mu \nabla^2 u \quad (1)$$

$$\nabla \cdot u = 0 \quad (2)$$

Where u , p , ρ and μ are the velocity vector, pressure, density and dynamic viscosity, respectively. All external forces are represented under F and it is included gravitational, centrifugal and Coriolis forces.

$$F = \rho g + F_{ce} + F_{co} = \rho(g + |\omega|^2 r - 2\omega \times u) \quad (3)$$

Where g , r , and ω are gravity, radial position vector of a point and angular velocity, respectively. The volumetric flow rate is determined through the following equation.

$$Q = VA \quad (4)$$

$$V = r\omega \quad (5)$$

Where V and A are velocity along the channel direction and cross section area of the channel, respectively. Capillary valve works based on capillary force resulting from the surface tension and capillary force per unit area is given by:

$$\Delta P_s = \frac{C\gamma \sin \theta}{A} \quad (6)$$

Where C , γ , θ and A are associated contact line length, the surface tension of the fluid, the contact angle and the cross section area of the microchannel respectively. The driving force of centrifugal is calculated from the following equation:

$$\Delta P_c = \rho \omega^2 (R_2 - R_1) \left(\frac{R_2 + R_1}{2} \right) = \rho \omega^2 \cdot \Delta R \cdot \bar{R} \quad (7)$$

Where R_1 and R_2 are distances of the liquid level in inlet and outlet from the COR of the CD. For the simplest condition that the reservoirs are equal and they contain the same amount of liquid, the liquid start flowing in a microchannel when the centrifugal force overcomes the capillary force. The frequency at this moment is called burst frequency and it can be determined from the following equation:

$$\Delta P_s = \Delta P_c \quad (8)$$

$$f_b = \left(\frac{\gamma \sin \theta}{4\pi^2 \rho \cdot \Delta R \cdot \bar{R} \cdot d_H} \right)^{1/2} \quad (9)$$

The latest derivation (equation 9) is based on reservoir equality and the liquid inside it [6].

III. METHODOLOGY

In this study, a microfluidic ELISA CD platform which gives the flow sequences with the diverse volume reservoirs was designed using AutoCAD 2008. This design performs an ELISA in an immunoassay application. The volumetric capacity of reservoir was adopted from the conventional dengue ELISA diagnostic protocol. The reservoirs and microchannel specifications are illustrated in Table 1.

For this particular experiment, the CD composed of 2 layers: the substrate layer is made of Poly Methyl Methacrylate (PMMA) (Enigma Marketing Sdn Bhd, Malaysia) and the top layer is laminated with an Adhesive Sealing Films (ASF) or thermal seal (Sigma-Aldrich, Germany). The PMMA Substrate CD consists of 7 reservoirs: waste (1), detection (2), serum (3), conjugate (4), washing solution (5), substrate (6) and stopping solution (7) as shown in Figure 1.

Table 1. MICROFLUIDIC DISC DESCRIPTION

Reservoirs			Microchannels		
Iter m No.	Name	Volume (10^{-5} mm^3)	Depth (mm)		Width (mm)
1	Waste	800			
2	Detection	150	2.3	0.150	300
3	Serum	100	2.3	0.150	600
4	Conjugate	100	2.3	0.150	400
5	Washing	300	2.3	0.150	300
6	Substrate	150	2.3	0.150	300
7	Stopping	100	2.3	0.150	300
	Venting channel			0.150	300
	Infusion channel			0.150	300

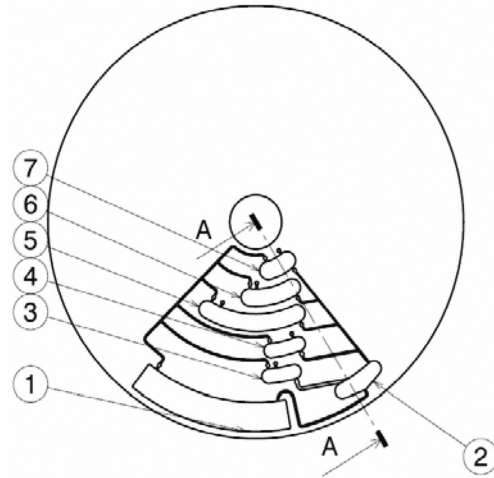


Fig. 1 Microfluidic ELISA disc design

A CNC Micromachine of Mikrottools DT110 (Mikrotool Pte Ltd, Singapore) with milling tool of 100 μm , 2 mm and drilling tool of 1mm are employed for engraving and patterning the PMMA substrate. A square PMMA sheet is carefully clamped and calibrated in order to get the optimum tolerances and distances on the CNC machine as shown in Figure 2.

The cleaned fabricated PMMA substrate is then laminated by an ASF as the top layer to prevent the liquid from spattering. There are 2 holes at each of the reservoir: air and loading holes (Figure 1). Air venting microchannel hole is connected to each of the reservoir from the top to avoid air pressure and to let the liquid to flow in the microchannel at the bottom of each reservoir. Each reservoir was filled with colour liquid via the loading hole in the disc which it is connected to the reservoir through a microchannel. To generate a barrier at the reservoir, a capillary force is introduced in front of the liquid at the reservoir. Therefore, all the microchannels are equipped with 500-millimeter-radius expansion to control the flow time with rotational speed.

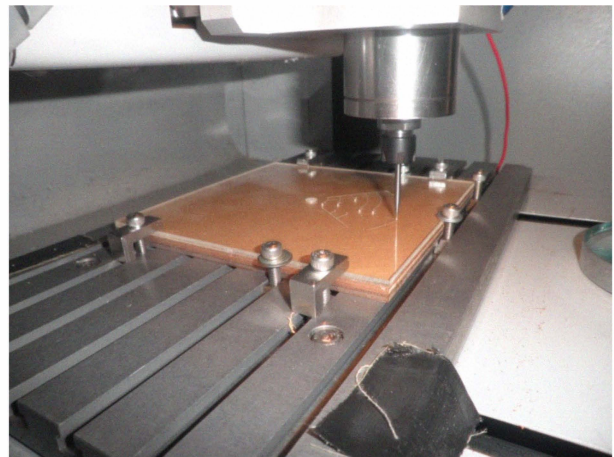


Fig. 2 Microfluidic disc fabrication by micromachining, University of Malaya

The final ELISA CD product is placed on a CD spin stand test system for testing the fluid flow sequence. The CD spin stand test system consist of a brushless DC servo motor mounted on a transparent PMMA based platform, a spin test controller unit to control the rotational speed of the motor and visualization system to capture the image of the detection which is interfaced with a computer (Figure 3). The rotational speed of the motor is set by the controller unit equipped with software (Labview) and a user can specify the rotational speed from 0 to 9200 rpm (nominal speed) for the motor. In large scale of number, the brushless DC servo motor consumes much less energy than conventional biomedical equipments and it leads to reduce the energy consumption for bio-analysis and microbiological purposes. In this study, the visualization system consists of a Charged Coupled Device (CCD) camera macro lens, lighting (either strobe light or LED and a laser tachometer. One microscope is adjusted to the system for image processing and more analytical purposes. Lighting is the most crucial and challenging factors to capture a clear image especially during rotational at high frequency. The CD spin test and visualization system for measuring the burst frequency is shown in Figure 3.

The CCD camera provides a good visualization system for the CD spin test and this is very important to capture and record the image of the liquid flow [21].

Equation 9 was employed to estimate the burst frequency of the microvalves in the CD even for the reservoirs with different volumes. The theoretical calculations will reflect the approximate estimation of the burst frequency this estimation provides the starting guideline value for finding the burst frequencies during the actual experiment.

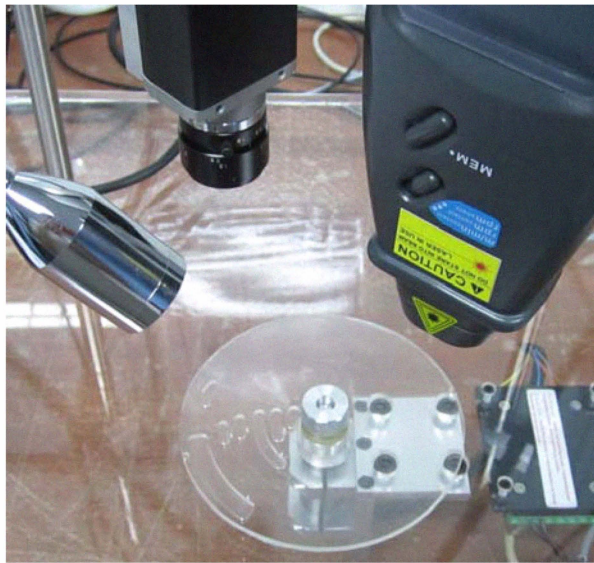


Fig. 3 CD spin test and Visualization system for monitoring the burst frequency

IV. RESULT AND DISCUSSION

Table 2 illustrates the estimation and experimental burst frequency for the valves in the CD. The experimental results show that the an average error for the burst frequency of 9.4% as compared to the calculated values obtained from equation 9.

From table 2, it seems that the deviation from the estimated burst frequency may be related to the hydrostaticity property of material. Also, the only different between this study design and the design for the (9) is the inequality of the reservoir volume. To date in the microfluidic research, it is assumed that the effect of volume and volumetric forces is negligible for application of centrifugal and capillary forces in Microsystems. However, the burst frequency is proportion to volume with the power of 0.5 in the (9). To investigate the correlation of the burst frequency (RPM) between equation 9 and the actual experimental result, a regression graph was plotted as in Figure 4. Figure 4 shows the experimental frequencies (f_{exp}) and estimated frequencies (f_{est}) for the 5 reservoirs excluding the waste chamber of the CD.

Equation 10 was derived from the regression graph in Figure 4. Equation 10 gives the relation between f_{exp} and f_{est} , shows that dependency of burst frequency to the volume can cause error range from 1% to 19% in the result when the burst frequency were calculated with the (9). Best fitting regression graph. X-axis is the experimental and Y-axis estimated burst frequency results equation 10.

$$f_{exp} = -0.0005f_{est}^2 + 1.474f_{est} \quad (1)$$

Table II. BURST FREQUENCY OF THE VAVLES

Reservoir	Volume (ul)	Calculated speed (rpm)	Measured speed (rpm)	Error (%)
Serum	80	599	733	18
Enzyme	80	678	700	3
Washing solution	250	905	967	6
Substrate	150	1044	1050	1
Stopping solution	80	1251	1050	19

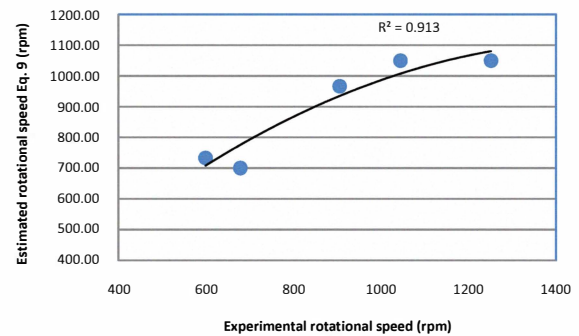


Fig. 4 Estimated rotational speed vs Experimental rotational speed for determining the burst frequency

From the experiment, it was realized that the output of visualization system is very sensitive to the lighting whereas using the top light or back light changes quality of the images significantly. For high rotational speeds (more than 50 Hz) lighting is a major problem. Lighting configuration can be improved by placing a white light diffuser at the top or bottom of the CD.

The simplicity of this project is using the fabrication method of 2 layer-CD. As known, the common method for CDs fabrication are by lithography or CNC micromachining. Lithography needs numerous expensive equipment and materials, while a 5 or 3 layer-CD can be generated by very tedious, time consuming process of CNC micromachining. But using 2-layer CD, it reduces the thickness, weight and material of the CD. The weight can be important parameter for choosing a motor for spinning system. Whatever the CD is lighter, smaller motor can be used. By applying this method required material reduces from 3 discs to a disc.

V. CONCLUSION

This paper has discussed the basic design, fabrication, analysis and experiment of centrifugal force for microfluidic ELISA on a compact-disc (CD) platform. This paper suggested that it is possible to use the 2-layer CD instead of 3 or 5-layer CDs to reduce the consumption of materials, weight of CD and consequently energy consumption of system. The inspiring application of the CD for the protocol of dengue diagnostic ELISA dictated to consider different volume for different reservoirs. Deviation of the measurements from the estimated burst frequency proved dependency of burst frequency to the volume of liquid in the reservoirs. The future work is to focus and understand on the volume effect toward the burst frequency algorithm.

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